PRILIMINARY PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF A NOVEL POLYHERBAL FORMULATION

First author- Dr. K Athira Mohan
Final year PG Scholar Department of Rasashastra and Bhaishajya Kalpana
KAHER Shri B.M. Kankanawadi Ayurveda Mahavidyalaya, Shahapur, Belagavi-590003, India.

Second and Corresponding author*- Dr. R. S. Hiremath MD. PhD Professor &HoD
Department of Rasashastra and Bhaishajya Kalpana
KAHER Shri B.M. Kankanawadi Ayurveda Mahavidyalaya, Shahapur, Belagavi-590003, India.

Third author- Dr. Revanna. V. Jambagi
Final year PG Scholar Department of Rasashastra and Bhaishajya Kalpana
KAHER Shri B.M. Kankanawadi Ayurveda Mahavidyalaya, Shahapur, Belagavi-590003, India.

Fourth author-
Dr. Rashmi
Final year PG Scholar Department of Rasashastra and Bhaishajya Kalpana
KAHER Shri B.M. Kankanawadi Ayurveda Mahavidyalaya, Shahapur, Belagavi-590003, India.

ABSTRACT:

Background: Traditional healing methods that are not professionally documented or organized contain formulations having combinations of drugs; one such formulation is mentioned in the article. There is a dire necessity to establish their activities. **Objective**: The purpose of the study was to examine the phytochemicals present and the antioxidant activity of the polyherbal formulation. **Materials and Methods*:*** The polyherbal formulation contains *Shashasruthi (Emelia sonchifolia Linn.), Haridra (Curcuma longa Linn.), Yashtimadhu (Glycyrrhiza glabra Linn.), Madhuchishta (Cera Alba), Narikela Taila (Cocos nucifera Linn.), and Karpoora (Cinnamomum camphora).* The *Lepa* was prepared according to SOP and subjected to physicochemical, phytochemical analysis and antioxidant activity. The in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to carry out the antioxidant investigation. **Results**: The results of the study demonstrated that Lepa possesses phytoconstituents, i.e., tannins, flavonoids, polyphenols, etc., and has potential antioxidant activity. **Conclusion**: Thus, the *Lepa* have good therapeutic potential as natural antioxidants and considering the phytochemicals present, lepa might be used in *Sadyo Vrana* as indicated.

Keywords: Phytochemical analysis, antioxidant activity, DPPH assay, polyherbal formulation, *sadyo vrana*, *lepa.*

INTRODUCTION

Traditional healing practices, which are age-old since time immemorial, have contributed extensively to the field of medicine. As these have not been properly documented since then, their usage and results are not accepted by the world. It's high time that these

preparations should be examined and the data saved in order to bring forth their actual potential. The polyherbal preparation is in the form of *Lepa* and was prepared out of *Shashasruthi (Emelia sonchifolia Linn.), Haridra (Curcuma longa Linn.), Yashtimadhu (Glycyrrhiza glabra Linn.), Madhuchishta (Cera Alba), Narikela Taila (Cocos nucifera Linn.), and Karpoora (Cinnamomum camphora).*

An antioxidant is any substance that stops or reduces oxidative damage to a target molecule. When present at low quantities relative to oxidizable substrates, a class of chemicals known as antioxidants, which are often oxidized themselves, significantly inhibit or postpone oxidative processes. Various strategies, such as electron donation (as reducing agents), metal ion chelation (which eliminates potential free radicals), and antioxidant sparing (co-antioxidants), can be used by antioxidants to influence biological systems.

The burden of free radicals can be lessened by antioxidants because of their ability to absorb, reduce, and stabilize free radicals. The primary attribute of an antioxidant is its capacity to capture free radicals. By scavenging free radicals like peroxide, hydroperoxide, or lipid peroxyl, antioxidant substances such as phenolic acids, polyphenols, and flavonoids prevent the oxidative processes that cause degenerative illnesses.

The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is frequently used to test compounds' potential to act as hydrogen donors or free radical scavengers and to assess antioxidant activity, provides a quick, easy, and affordable way to determine the antioxidant capacity [1].

Further in the study, phytochemical, physicochemical analysis, and antioxidant activity were carried out.

MATERIALS AND METHODS

Raw Drugs:

All the raw drugs were procured from KLE Ayurveda Pharmacy, Khasbag, Belagavi, and authenticated. Phytochemical, physicochemical analysis and antioxidant activity were carried out at the AYUSH-approved Drug Testing Laboratory at KAHER's SHRI B M Kankanawadi Ayurved Mahavidyalaya, Belagavi.

Preparation of Polyherbal Formulation:

Preparation of *Churna*: *Haridra* and Yashtimadhu were made into *Churna* using a pulverizer and passed through 120 mesh size separately [2].

Fig 1-*Haridra Churna*

Fig 2-*Yashtimadhu Churna*

Fig 3- Mixture

Preparation of *Swarasa*: 10ml *Shashasruthi swarasa* was prepared according to SOP of *swarasa kalpana* [3].

Fig 4- *Shashasruthi* Plant

Fig 5- Trituration

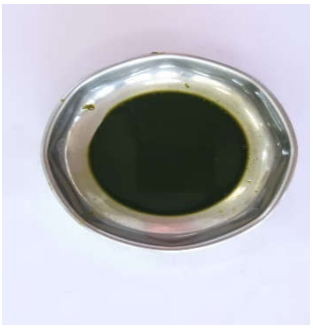


Fig 6- *Swarasa*

Preparation of the Formulation:

Table no. 1: Ingredients and Proportion of polyherbal formulation

SL No	Ingredients	Proportion
1	Haridra	1 Part
2	Yashtimadhu	1 Part
3	Shashasruthi	2 Part
4	Narikela Taila	2 Part
5	Karpoora	1 Part
6	Madhuchishta	1 Part

5 g of each of the *churnas* were made into a homogeneous mixture by triturating in *Khalwa Yantra* at the Department of Rasashastra and Bhaishajya Kalpana, KLE SHRI B. M. Kankanawadi Ayurveda Mahavidyalaya, Shahapur Belagavi. 10 ml *Shashasruthi swarasa* and 10 ml *Narikela Taila* were added simultaneously. The homogeneous mixture is then triturated with 5g of powdered *Karpoora*. 5g of *Madhuchishta* is melted, filtered, and added to the mixture and triturated well until *Lepa* consistency. It took 7-8 minutes for the preparation, and 35.7g of total *Lepa* was obtained. (Figure-7)

Haridra and Yashtimadhu

Adding *Swarasa*

Narikela Taila

Fig 7- Demonstration of Lepa preparation

Preparation of Extract: Polyherbal formulation was extracted using both water and alcohol solvents individually. Macerate 5g of *lepa* dissolved in 100ml distilled water and methanol for aqueous and alcoholic extracts, respectively, in beakers for 24 hours, shaking frequently for 6 hours and allowing to stand for 18 hours [4].

Phytochemical Analysis [5]:

Every analysis was conducted in accordance with the Indian Ayurvedic Pharmacopoeia.

DPPH ASSAY [6]:

Chemicals required: ascorbic acid, plant extract, methanol, DPPH chemical, and aluminum foil.

Principle: DPPH is an organic nitrogen radical capable of accepting the hydrogen from the sample(s). DPPH is purple in colour and converts to yellow by the formation of the DPPH (upon conversion from radical to the compound). The reduction of UV absorption in the DPPH in both the test and control samples is a measure of the antioxidant.

Preparation of Working Solutions and Procedure:

Ascorbic acid was used to make the calibration curve by dissolving it in methanol and then diluting it to 6.25-200 µg/ml of serial concentrations. Stock solution of extracts (1 mg/ml) was prepared with methanol and further diluted to serial concentrations, e.g., 6.25-200 µg/ml.

Reaction solutions of 5 ml contained sample extract serial concentrations / (ascorbic acid standard serial concentrations) to which DPPH dissolved in methanol was added and mixed well. The blank contained only DPPH dissolved in methanol. Incubate the tubes for 30mins in the darkroom.

Absorbance of blank, sample, and standard solutions was measured at 517 nm with a Shimadzu UV-1800 spectrophotometer. Calibration curves using ABSORBANCE vs. CONCENTRATION of sample extract serial concentrations and ascorbic acid standard serial concentrations were prepared, and the percentage of radical scavenging activity in the sample was determined by using the following equation.

$$\text{Percentage (\%) of radical scavenging activity} = \frac{\text{Abs of the blank-Abs of the sample X 100}}{\text{Abs of the blank}}$$

*Abs-Absorption

RESULTS

Table 2: Phytochemical Analysis of Polyherbal Formulation

Tests	Vrana Ropaka Kalpa	
	Alcohol	**Water**
Carbohydrates	Negative	Positive
Reducing sugar	Positive	Positive
Monosaccharides	Negative	Negative
Pentose sugar	Negative	Negative
Non-reducing sugar	Negative	Negative
Hexose sugar	Negative	Negative
Protein	Negative	Negative
Amino acids	Negative	Negative
Steroids	Positive	Negative
Flavonoids	Negative	Positive
Alkaloids	Negative	Negative
Tannins	Positive	Positive
Saponins	Negative	Positive
Cardiac Glycosides	Negative	Negative
Anthraquinone glycosides	Negative	Negative

Based on the obtained results, the prepared Polyherbal formulation contains Tannins, Flavonoids, Steroids and Saponins which aids to wound healing activity.

Table 3: Physicochemical Analysis of Polyherbal Formulation

Tests		**Results**
Ash values	Acid Insoluble ash	0.00%
	Water soluble ash	0.816%
Extractive Values	Water soluble extractive	5.603%
	Alcohol soluble extractive	26.707%
pH	5% Solution	6.07

The results indicate 0.00% of acid insoluble ash,0.816% of water-soluble ash,5.603% of water-soluble extractive,26.707% of alcohol soluble extractive with a pH of 6.07.

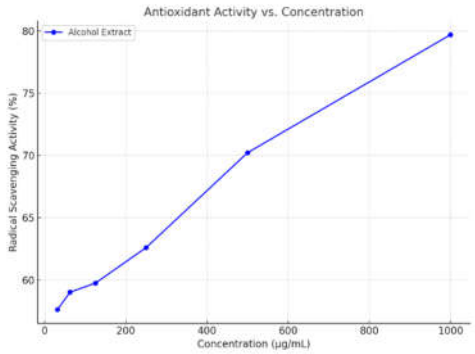
Table 4: Antioxidant Activity of Polyherbal Formulation using DPPH Assay compared to Ascorbic acid

Alcohol extract	
Concentration µg/mL	**% Radical scavenging activity**
31.25	57.62
62.5	59.02

125	59.75
250	62.60
500	70.22
1000	79.69

Note: Results are mean of three readings

The results show antioxidant activities with increasing efficacy at higher concentrations.



Graph 1: LC50 graph for the alcohol extract, showing the relationship between concentration and radical scavenging activity.

DISCUSSION:

According to WHO traditional herbal medicine is a major source in developing countries for health care [7] and a large number of synthetic medicines are derived or based on medicinal plants [8]. There is a need of the hour to establish their potential by screening their bioactive compounds and their benefits.

Phytochemical analysis: Depending on the kind of solvent, alcohol extract showed presence of reducing sugar, steroids and tannins. Whereas water extract had presence of carbohydrates, reducing sugar, flavonoids, and saponins.

The bioavailability of additional active ingredients in a plant extract can be impacted by the kind and quantity of reducing sugars present [9]. In the results obtained it shows the presence of reducing sugar which indicates the bioavailability of the formulation.

Steroids present in the plant attributes to antiviral, antibacterial, anti-inflammatory activities [10].

Many plants include a class of polyphenolic chemicals called tannins. They provide a variety of functions in plants and are widely used in industry, medicine, and human health. Their astringent qualities aid in tissue healing and coagulation and reduce the damaging effects of dangerous proteins or alkaloids by binding with them. Tannins probably support the formulation's ability to promote tissue repair and manage oxidative stress by having antioxidant and wound-healing qualities.

Presence of carbohydrates in the formulation gives the actions like antioxidant, antiviral and anti-inflammatory activities [11].

A varied class of polyphenolic chemicals derived from plants; flavonoids are well-known for their bioactive qualities. According to the study, flavonoids help with antioxidant activity by promoting wound healing, lowering oxidative stress and anti-inflammatory effects by promoting the healing of damaged tissue, and antimicrobial action by guarding against ulcers or wound infections.

Saponins are a family of naturally occurring compounds found in many plants. Their capacity to produce foam when shaken with water is what gives them their soap-like qualities. In regard with the study, saponins aid in preventing wound infections. The properties of surfactants and foaming make it easier to clean wounds. Enhance tissue repair and lessen inflammation by promoting healing and reducing inflammation.

Physico-chemical analysis: Acid-insoluble ash (AIA) specifically indicates the presence of silica-based impurities, such as sand, soil, or other insoluble materials that might be present due to poor handling or processing of raw materials. It ensures the formulation or raw material meets established purity standards, helps differentiate between genuine and adulterated samples [12]. Excess AIA could indicate harmful levels of contaminants, affecting the safety of the product for human use. It can be ensured that the herbal product is free of high amounts of inorganic contamination by keeping an eye on acid-insoluble ash, which will improve its overall safety and effectiveness. In the prepared polyherbal formulation, AIA shows 0.00%, which indicates the purity and safety of the formulation.

Water-soluble ash is a crucial indicator for verifying the quality and purity of herbal formulations and raw materials. It guarantees that the product is free of impurities and satisfies all necessary requirements, making it safe and efficient for use in medicine or as a food. In the preparation it shows 0.86%, which is indicative of the fact that the formulation was made with proper SOP and hygiene. The number of water-soluble phytochemicals, including alkaloids, glycosides, tannins, flavonoids, saponins, and some vitamins, that are present in the sample can be estimated using a water-soluble extract, and the same goes for an alcohol-soluble extract.

pH of 6.07 indicates basic nature of formulation. A pH more than 6.5 is indicative of slow healing and high risk of infections [13]. In contrast a pH in between 5.5-6.5 is indicative of favourable environment for cell proliferation, increased oxygen availability and antimicrobial activity [14].

Anti-oxidant activity: In organisms including humans, reactive oxygen species (ROS) and free radicals are produced during metabolic and immune system function [15]. The excess free radicals are responsible for the generation of various diseases [16]. The antioxidants neutralize the effect and action of free radicals and prevent the development of various diseases [17]. As the water extract of the formulation didn’t show much results on antioxidant activity, Antioxidant tests demonstrated significant radical scavenging activity, particularly in the alcoholic extract, with increasing efficacy at higher concentrations.

CONCLUSION:

On analysing the Phytochemicals, it was found that the polyherbal formulation contained Flavonoids, Tannins and Saponins. Physicochemical analysis shoots lower range of adulterations and contamination in the product which indicates that the preparation was done with proper hygiene and the raw materials were of standards. Based on the above results, polyherbal formulation exhibits antioxidant properties that could have therapeutic applications in managing oxidative stress-related conditions, including wound healing.

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